REMARKS

After entry of this amendment, claims 1-15 are pending. The claims have been amended without prejudice or disclaimed to correct the antecedent basis and to better comply with U.S. practice. Support is found, *inter alia*, in the original claims. No new matter has been added.

Claims Rejections – 35 USC § 103

Claims 1, 4-7, 12-13 and 15 are rejected under 35 USC § 103(a) as being obvious over Henikoff in view of Zaccolo *et al.* (hereinafter "Zoccolo"). Claims 2, 8-11 and 14 are rejected under 35 USC § 103(a) as being obvious over Henikoff in view of Zaccolo, further in view of Krokan *et al.* (hereinafter "Krokan") and Short *et al.* (hereinafter "Short"). Claim 3 is rejected under 35 USC § 103(a) as being obvious over Henikoff in view of Zaccolo, further in view of Krokan and Short, and further in view of Lutz *et al.* (hereinafter "Lutz") and Cosstick *et al.* (hereinafter "Cosstick"). Applicants respectfully disagree and traverse the rejection.

The Examiner bears the initial burden of establishing *prima facie* obviousness. See *In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). To support a *prima facie* conclusion of obviousness, the prior art must disclose or suggest all the limitations of the claimed invention. See *In re Lowry*, 32 F.3d 1579, 1582, 32 USPQ2d 1031, 1034 (Fed. Cir. 1994); see also *Ex parte Alexander*, 86 USPQ2d 1120, 1122 (BPAI 2007) (where the Board reversed the obviousness rejection in part because the Examiner had not identified all the elements of the claim). Here, the claimed limitations (process steps) are not suggested by the references cited in proper combination. As explained below, neither reference shows creation of single-stranded fragments with different length as in step (i) of the claimed process, and no reference shows creation of an ordered series of mutants having the same length as the master sequence, the product of steps (i)-(iv) of the claimed process.

The Examiner characterizes Henikoff as disclosing a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) of n base-pairs comprising the steps of (i) and (iv) as recited in claim 1 of the instant application. According to the Examiner, step (i) as recited in claim 1 can be achieved by practicing steps 1 to 3 as outlined in Figure 1 of Henikoff at page 2962. Additionally, the Examiner alleges that step (iv) can be achieved by using appropriate helper phage to produce the single-stranded sequence of both the (+) and (-) strands and synthesizing a (-)-strand by using the (+)-strand as a template as taught by Henikoff

at page 2966. The Examiner acknowledges that Henikoff does not teach steps (ii) of claim 1, but relies on Zoccolo for such teaching. Applicants disagree that the invention as claimed would have been obvious from the cited references.

As described in the specification and recited in the claims, the present application relates to a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) of n base-pairs by first creating a collection of **single-stranded fragments** of the (+)-strand of the master sequence, wherein each single-stranded fragment has the same 5'-terminus but different length due to the deletion in the 3'-terminus. See, e.g., Specification at page 1, lines 27-30, page 3, lines 20-34, and the end products of the scheme shown in Figure 1a. Thus, the collection of polynucleotide fragments to be used for introducing at least one universal or degenerate nucleotide at the 3'-terminus in step (ii) is a collection of **single-stranded fragments**. It is clear that this limitation of the claimed process is not taught in Henikoff.

As illustrated in Figure 1 of Henikoff, the collection of polynucleotide fragments generated by the disclosed ExoIII deletion is a collection of **double-stranded fragments**. As the Examiner noted, the complete circular strand, the "(-)-strand" according to the Examiner, is **hybridized with** the various 3' deletions of the "(+)-strand" generated by the ExoIII digestion. See Office Action at page 3. After digesting with ss nuclease, a collection of double-stranded polynucleotide fragments is generated, each of which has the same 5'-terminus but various length at the 3'-terminus due to the deletion. These double-stranded fragments are then repaired and ligated into circular plasmids for E. coli transformation. Thus, it is clear that the so-called "(+)-strand" fragments in Henikoff's method never exist in a status of "single-stranded fragments" as required by step (i) of claim 1.

Furthermore, it is respectfully submitted that the Examiner's reliance on Henikoff's description at page 2966 for teaching step (iv) of the claimed process is misplaced. As required by steps (iii) and (iv) of the claimed process, the **full-length** of the master sequence must be synthesized. This is further illustrated in Figure 4, where a (-)-strand complementary to the full length of the (+)-strand is synthesized in step (iv). Conversely, the use of helper phage *Mike* or M13K07 in Henikoff as discussed at page 2966 is to generate a single-stranded template, either the (+)-strand or the (-)-strand, for polymerase extension (*i.e.* step 2 in Figure 1 at page 2962), or for sequencing the resulting deletion clones. Because of the existence of a nick after the

polymerase extension of step 2 in Henikoff, it is clear that the full-length of the template strand (master sequence) is not synthesized. As to the use for sequencing, it is also clear that the full-length of the template strand (master sequence) would not be synthesized from the resulting deletion clones.

Additionally, as illustrated in Figure 1 at page 2962, Henikoff's nested deletion method produces polynucleotide molecules of different length, all of them are **shorter** than the master sequence. In contrast, the process of the present invention produces mutated polynucleotide molecules having the **same length** as the master sequence. See claim 1, step (iii). Therefore, Applicants respectfully submit that Henikoff does not teach or suggest the steps of the claimed process as alleged by the Examiner.

Zoccolo does not remedy the deficiencies of Henikoff. Zoccolo teaches an approach to random mutagenesis of DNA based on PCR amplification in the presence of a mixture of triphosphates of nucleoside analogues. The PCR mutagenesis is to be performed by amplifying a target DNA fragment in the presence of four normal dNTPs (*i.e.* dATP, dGTP, dCTP, and dTTP) and the nucleoside analogues, dPTP and/or 8-oxodGTP. Accordingly, more than one nucleoside analogue is incorporated into the newly amplified DNA strand in a random fashion, which results in more than one nucleotide substitution scattered over the whole polynucleotide sequence. This is further evidenced by Figure 5 at pages 595-596 of Zoccolo, in which each amplified sequence contains more than one nucleotide substitution randomly scattered over the whole molecule. In contrast, the process of the present invention is aimed to introduce nucleotide substitution in only one location of any given polynucleotide molecule. This is achieved by step (ii) of the process as recited in claim 1, where the universal or degenerate nucleotide is introduced at the 3'-terminus of the (+)-strands produced in step (i) and not randomly incorporated by PCR amplification of a DNA template. The use of PCR amplification to introduce the nucleotide substitution in Zoccolo further distinguishes the method taught therein from the process of the present invention. Accordingly, it is respectfully submitted that Zoccolo does not teach or suggest step (ii) of the claimed process as recited in claim 1.

Neither Henikoff nor Zoccolo, alone or in combination, teach or suggest a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) wherein a collection of single-stranded fragments with different length of the master sequence is first

generated. Neither Henikoff nor Zoccolo, alone or in combination, teach or suggest a process for the mutagenesis of a double-stranded polynucleotide sequence (master sequence) wherein each mutant sequence obtained thereform has the same length as the master sequence and has nucleotide substitution occurred only in one single location in any given polynucleotide molecule. Henikoff and Zoccolo, alone or in combination, do not disclose or suggest the claimed series of steps. Even if combinable, there is no teaching of a step using different length of single-stranded polynucleotide sequences. Even if combinable, the combined teaching suggests, at the most, creation of a series of shortened mutants, and not a collection of molecules with the same length as the master sequence produced by the claimed process.

Furthermore, it is well established that under 35 U.S.C. § 103 the Examiner must consider the reference as a whole, including portions that teach away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). In addition, the Examiner cannot selectively pick and choose from the disclosed parameters without proper motivation as to a particular selection. The mere fact that a reference may be modified to reflect features of the claimed invention does not make the modification, and hence the claimed invention, obvious unless the prior art suggested the desirability of such modification. *In re Mills*, 916 F.2d 680, 682, 16 USPQ2d 1430 (Fed. Cir. 1990); *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992). "[A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art . . . it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements ***in the way the claimed new invention does.***" See *KSR International Co. v. Teleflex Inc.*, 1741 82 USPQ2d 1385, 1396 (2007) (emphasis added). Thus, it is impermissible to simply engage in a hindsight reconstruction of the claimed invention where the reference itself provides no teaching as to why the applicant's combination would have been obvious. *In re Gorman*, 933 F.2d 982, 987, 18 USPQ2d 1885, 1888 (Fed. Cir. 1991).

When considering Henikoff as a whole, Henikoff teaches a method to produce nested deletions in polynucleotides. The "mutagenesis" produced by Henikoff's method therefore is deletion, not point mutations. On the other hand, Zoccolo teaches random mutagenesis of DNA by PCR amplification in the presence of nucleoside analogues. The "mutagenesis" produced by

Zoccolo's method therefore is point mutations, not deletion. Because Henikoff and Zoccolo teach different concept of mutagenesis with different series of steps, there is no motivation to combine Henikoff and Zoccolo but for an impermissible hindsight reconstruction of the claimed invention.

Because motivation to combine the references is lacking, and because all the limitations of the claims are not taught, Henikoff and Zoccolo, alone or in combination, do not render obvious the subject matter of the independent claims or the claims dependent therefrom. *See In re Fine*, 837 F.2d 1071, 1076 (Fed. Cir. 1988) (holding that if an independent claim is nonobvious then any claim dependent therefrom is nonobvious).

For at least the above reasons, reconsideration and withdrawal of the rejections is respectfully requested.

CONCLUSION

For at least the above reasons, Applicants respectfully request withdrawal of the rejections and allowance of the claims. If any outstanding issues remain, the Examiner is invited to telephone the undersigned at the number given below.

Accompanying this response is a petition for a three-month extension of time to and including October 9, 2008 to respond to the Office Action mailed April 9, 2008 with the required fee payment. No further fee is believed due. However, if any additional fee is due, the Director is hereby authorized to charge our Deposit Account No. 03-2775, under Order No. 12810-00231-US from which the undersigned is authorized to draw.

Respectfully submitted,

By _____

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